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# Cardiovascular Autonomic Function Is Associated With (Micro-)Albuminuria in Elderly Caucasian Subjects With Impaired Glucose Tolerance or Type 2 Diabetes

## The Hoorn Study

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**OBJECTIVE** — To determine whether impaired cardiovascular autonomic function correlates with albuminuria in an age-, sex-, and glucose tolerance-stratified sample of an elderly (50–75 years of age) Caucasian population and to determine whether this association is independent of other determinants of albuminuria.

**RESEARCH DESIGN AND METHODS** — We studied 536 subjects, 256 with normal glucose tolerance, 143 with impaired glucose tolerance (IGT), and 137 with type 2 diabetes. Microalbuminuria was defined as an albumin-to-creatinine ratio of  $\geq 3.0$  and  $\leq 30$  mg/mmol in an early morning urine sample. We used the deep-breathing test and the lying-to-standing test to obtain 4 measurements of cardiovascular autonomic function: 1) the heart rate (HR) variability during deep breathing, 2) the maximum HR within 15 s after standing up minus the mean HR before standing, 3) the maximum R-R interval between 15 and 30 s after standing up divided by the minimum R-R interval within 15 s after standing up, and 4) the systolic blood pressure in response to standing up. These 4 measurements were summarized in a single cardiovascular autonomic function score (CAFS).

**RESULTS** — A total of 38 subjects with microalbuminuria and 3 subjects with macroalbuminuria ( $>30$  mg/mmol) were grouped as having albuminuria. In bivariate analyses, albuminuria was associated with age, waist-to-hip ratio, systolic and diastolic blood pressure, calculated glomerular filtration rate, and glucose tolerance status. The mean CAFS was higher in subjects with versus without albuminuria (7.5 vs. 5.9,  $P < 0.001$ ). Multiple logistical regression analyses revealed that the CAFS was independently associated with albuminuria in subjects with IGT or type 2 diabetes with an odds ratio (95% CI) of 1.19 (1.02–1.39) per point increase in the CAFS.

**CONCLUSIONS** — Impaired cardiovascular autonomic function is independently associated with (and thus a possible contributor to) the presence of albuminuria in subjects with IGT or type 2 diabetes.

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**Abbreviations:** CAFS, cardiovascular autonomic function score; CVD&D, cardiovascular disease and/or drugs;  $\Delta HR_{diff}$ , the difference between intrabreath maximum and minimum heart rate averaged over 6 breaths; HR, heart rate; IGT, impaired glucose tolerance;  $L \rightarrow S \Delta BP_{sys}$ , systolic blood pressure after standing up minus the supine systolic blood pressure;  $L \rightarrow S \Delta HR_{max}$ , maximum heart rate within 15 s after standing up minus the mean heart rate during 1 min before standing;  $L \rightarrow S_{max/min ratio}$ , the maximum R-R interval between 15 and 30 s after standing up divided by the minimum R-R interval within 15 s after standing up; NGT, normal glucose tolerance.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Increased urinary albumin excretion is a strong predictor of cardiovascular disease and mortality in patients with type 2 diabetes as well as in nondiabetic subjects. The pathophysiological explanation for this association is not entirely clear, but the risk marker albuminuria has been associated with the presence of several cardiovascular risk factors, including hypertension, dyslipidemia, poor glycemic control, a prothrombotic state, and generalized endothelial dysfunction (1).

Cardiovascular autonomic neuropathy is also strongly related to cardiovascular mortality in type 2 diabetes (2,3). Several studies have suggested that impaired cardiovascular autonomic function and increased urinary albumin excretion are related in patients with diabetes (4–8). Most of these studies have been done in type 1 diabetes. Similar studies in type 2 diabetes were relatively small or did not include subjects with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) for comparison with subjects with type 2 diabetes (9–14). In the present study, we investigated the relationship between cardiovascular autonomic function and albuminuria by using data from a large population-based study including subjects with NGT and IGT and type 2 diabetes. We were specifically interested in determining whether autonomic cardiovascular function is related to albuminuria independently of other factors that are known to be associated with albuminuria, including glucose intolerance and hypertension.

## RESEARCH DESIGN AND METHODS

### Subjects

The Hoorn Study is a cross-sectional investigation of glucose tolerance and other cardiovascular risk factors in a Caucasian population that was conducted from 1989 to 1992. A random sample of all men and

women 50–75 years of age was drawn from the municipal population registry of the town of Hoorn, the Netherlands. A total of 2,484 subjects participated (response rate 71%). An extensive cardiovascular investigation was performed in an age-, sex-, and glucose tolerance–stratified random subsample ( $n = 631$ , response rate 89%) as described in detail elsewhere (15). The Hoorn Study was approved by the Ethical Review Committee of the University Hospital Vrije Universiteit. Informed consent was obtained from all participants.

### Measurements

Height and weight were measured while subjects were barefoot and wearing light clothes only. Double readings of systolic and diastolic (Korotkoff V) blood pressure levels were obtained on 2 separate occasions in a sitting position. Actual hypertension was defined as mean systolic blood pressure  $\geq 160$  mmHg and/or mean diastolic blood pressure  $\geq 95$  mmHg with or without antihypertensive medication. IGT and type 2 diabetes were diagnosed according to the 1985 World Health Organization criteria applied to the mean of 2 standard oral glucose tolerance tests (16). Fasting and 2-h postload venous plasma glucose levels were determined with a glucose dehydrogenase method. Subjects with previously diagnosed type 2 diabetes treated with oral medication or insulin did not undergo a glucose tolerance test. We measured fasting serum levels of total cholesterol, HDL cholesterol, and triglycerides (enzymatic techniques); creatinine (modified Jaffé method); and total homocysteine and HbA<sub>1c</sub> (high-performance liquid chromatography). Glomerular filtration rate was estimated from serum creatinine using the formula described by Levey et al. (17). Albuminuria was measured in an early morning first voided urine sample. The presence of leukocytes was tested by light microscopy and scored as positive if  $>5$  leukocytes/high-power field were found. Albumin concentration was measured by rate nephelometry (Array Protein System; Beckman, Galway, Ireland) with a detection threshold of 6.2 mg/l and intra- and interassay coefficients of variation of 5 and 8%, respectively. Urinary creatinine was measured with a modified Jaffé method. A urinary albumin-to-creatinine ratio of  $\geq 3.0$  mg/mmol was taken as the lower cutoff value for microalbuminuria, and a ratio of  $>30$  mg/mmol was defined as macroalbuminuria. Urine samples were available for a total of 607

subjects. Subjects using an ACE inhibitor ( $n = 32$ ) were excluded to avoid misclassification of the dependent variable, which left 575 subjects for further analysis. Protein intake was calculated using a self-administered validated semiquantitative food frequency questionnaire (18).

A detailed cardiovascular history was obtained from all subjects and, if positive, was accepted only when confirmed in writing by the subject's general practitioner. Cardiovascular disease was defined as coronary artery, cerebrovascular, or peripheral artery disease. All subjects with cardiovascular disease and those using drugs affecting the cardiovascular system (including antihypertensive and antiarrhythmic drugs,  $\beta$ -blockers, diuretics, and vasodilators) were classified as positive for cardiovascular disease and/or drugs (CVD&D).

### Cardiovascular autonomic function tests

Both the deep-breathing test and the lying-to-standing test were performed by the same investigator. Subjects refrained from smoking and drinking coffee for at least 2 h before the tests. A light meal at least 1 h before the measurements was allowed. The tests were done in quiet surroundings with a room temperature between 19 and 22°C. After 10 min of rest in a supine position, the deep-breathing test was performed by breathing deeply for 1 min at a frequency of 6 breaths/min (5 s in, 5 s out). After 5 min of rest, the lying-to-standing test was performed. The subjects stood up as quickly as possible and remained standing for 2 min. During both tests, heart rate (HR) and blood pressure were registered using a computer-based data acquisition system. An electrocardiographic registration was obtained from bipolar chest leads. Beat-to-beat systolic and diastolic blood pressure levels were measured noninvasively on the right middle finger with the Finapres (Type BP2300; Ohmeda, Englewood, CO).

The following measures of cardiovascular autonomic function were used.

- $El_{HR_{diff}}$  (beats/min): the difference between intrabreath maximum and minimum HR averaged over 6 breaths,
- $L \rightarrow S\Delta HR_{max}$  (beats/min): the maximum HR within 15 s after standing up minus the mean HR during 1 min before standing,
- $L \rightarrow S_{max/min\ ratio}$  (dimensionless): the maximum R-R interval between 15 and 30 s after standing up divided by the mini-

mum R-R interval within 15 s after standing up, and

- $L \rightarrow S\Delta BP_{sys}$  (mmHg): the systolic blood pressure after standing up (mean between 1.5 and 2.0 min after standing) minus the supine systolic blood pressure (mean of 30 s).

The  $El_{HR_{diff}}$  and the  $L \rightarrow S\Delta HR_{max}$  predominantly reflect parasympathetic function, the  $L \rightarrow S_{max/min\ ratio}$  is the result of a vagal reflex to sympathetically mediated vasoconstriction, and the  $L \rightarrow S\Delta BP_{sys}$  is considered to reflect mainly peripheral sympathetic function. However, the specificity of these tests for the 2 different types of autonomic function is limited (19,20). Results were discarded if multiple nonsinus beats occurred during testing, if standing up took  $>10$  s, or if the recordings were technically unsuccessful for  $>1$  of the 4 measurements ( $n = 30$ ). Subjects with a history of neurological diseases or drug use known to influence autonomic nerve function (e.g., anti-Parkinson's drugs; phenytoin; antihistamines; and parasympatholytic, sympathomimetic, and parasympathomimetic drugs) were also excluded from the analysis ( $n = 9$ ), which left 536 subjects for final inclusion.

### Statistical methods

Routine bivariate tests were used to test for group differences between subjects with and without albuminuria. Correlations between the 4 autonomic function measures were calculated using Pearson's correlation coefficients, if required after log-transformation. Based on the 4 measures of cardiovascular autonomic function, a summary cardiovascular autonomic (dys)function score (CAFS) was constructed as follows. First, the results of each measurement were divided into quartiles. A subject was assigned 0 points if the result was in the most normal quartile (lowest values for the  $L \rightarrow S\Delta BP_{sys}$  test, highest values for the other 3 measures), 1 point if the result was in the second quartile, 2 points if the result was in the third quartile, and 3 points if the result was in the most abnormal quartile (highest values for the  $L \rightarrow S\Delta BP_{sys}$ , lowest values for the other measures). If all 4 measures were completed successfully, then the scores of each were added together. If 1 result was missing (44 of 536 subjects), then it was replaced by the median score (1.5 points). The result is a CAFS ranging from 0 (good) to 12 (poor). Multiple logistical regression analysis was used to identify independent corre-

lates of albuminuria. The presence of interaction effects was studied by entering product terms into the model.

**RESULTS** — Of the 536 subjects included, 256 had NGT, 143 had IGT, and 137 had type 2 diabetes. Subjects with microalbuminuria ( $n = 38$ ) and with macroalbuminuria ( $n = 3$ ) were grouped as having albuminuria. In the 3 categories of glucose tolerance, 4% of NGT, 7.5% of IGT, and 18% of type 2 diabetic subjects had albuminuria ( $P < 0.001$  by  $\chi^2$ ). Demographic and clinical data of subjects with and without albuminuria are shown in Table 1. Age, waist-to-hip ratio, BMI, blood pressure, glomerular filtration rate, and glucose tolerance were significantly related to the presence of albuminuria in bivariate tests. All 4 measures of cardiovascular autonomic function showed less favorable results in subjects with albuminuria.

Table 2 displays the difference in CAFS between subjects with and without albuminuria, with separate results given for substrata of glucose tolerance and actual hypertension. In the entire group, the CAFS was significantly related to albuminuria. This relationship did not vary greatly with glucose tolerance status or the presence of hypertension. No evidence existed of a threshold effect in the relationship between the CAFS and albuminuria (data not shown). As shown in Table 2, the CAFS itself was closely related to glucose tolerance; the means  $\pm$  SD CAFS was  $5.4 \pm 2.7$  in NGT,  $6.1 \pm 2.9$  in IGT, and  $7.1 \pm 2.8$  in type 2 diabetic ( $P < 0.001$  by analysis of variance) subjects.

In multivariate analysis, none of the individual associations between the 4 separate measures of cardiovascular autonomic function and albuminuria retained statistical significance after adjustment for potential confounders (data not shown). The CAFS, however, was independently related to the presence of albuminuria in the complete study group (Table 3). Multiple logistical regression analysis was also performed in strata of glucose tolerance. In these analyses, the IGT and the type 2 diabetic subjects behaved similarly regarding the association between the CAFS and albuminuria; the odds ratio (95% CI) (calculated as in model 2 of Table 3) for having albuminuria was 1.26 (0.97–1.64) for IGT and 1.22 (0.98–1.52) for type 2 diabetes. Because of this similarity—and its contrast with the results obtained for NGT—subjects with IGT and type 2 diabetes were

**Table 1—Demographic and clinical data of normoalbuminuric and albuminuric subjects**

	Normoalbuminuric subjects	Albuminuric subjects	P
<i>n</i>	495	41	—
Age (years)	63.7 $\pm$ 7.1	66.7 $\pm$ 6.0	0.009
Sex (M)	47	54	0.40
Waist-to-hip ratio			
Men	0.96 $\pm$ 0.07	0.97 $\pm$ 0.06	0.55
Women	0.87 $\pm$ 0.08	0.93 $\pm$ 0.05	<0.001
BMI (kg/m <sup>2</sup> )	26.9 $\pm$ 3.7	28.6 $\pm$ 4.8	0.008
Systolic blood pressure (mmHg)	136.7 $\pm$ 18.2	152.4 $\pm$ 20.2	<0.001
Diastolic blood pressure (mmHg)	81.9 $\pm$ 9.5	87.4 $\pm$ 12.0	0.001
Mean arterial blood pressure (mmHg)	100.2 $\pm$ 11.3	109.0 $\pm$ 13.1	<0.001
Actual hypertension	14	44	<0.001
NGT/IGT/type 2 diabetes	50/27/23	24/24/52	<0.001
HbA <sub>1c</sub> (%)	5.5 (2.7–15.2)	6.0 (4.5–12.1)	0.002
Total cholesterol (mmol/l)	6.7 $\pm$ 1.2	6.6 $\pm$ 1.2	0.68
HDL cholesterol (mmol/l)	1.25 (0.56–3.43)	1.10 (0.57–2.29)	0.08
Triglycerides (mmol/l)	1.5 (0.4–14.0)	1.8 (0.7–13.9)	0.12
Currently smoking (yes)	28	27	0.84
Protein intake (g $\cdot$ kg <sup>-1</sup> $\cdot$ day <sup>-1</sup> )	0.95 (0.29–2.38)	0.90 (0.31–1.80)	0.56
Homocysteine ( $\mu$ mol/l)	11.3 (4.9–49.6)	12.4 (6.1–77.5)	0.20
Glomerular filtration rate (ml/min)	71.5 $\pm$ 19.7	61.8 $\pm$ 18.6	0.003
Autonomic function measures			
EIHR <sub>diff</sub> (beats/min)	10.7 (1.9–42.7)	8.7 (2.5–29.4)	0.27
L $\rightarrow$ S $\Delta$ HR <sub>max</sub> (beats/min)	20.6 (6.5–46.4)	17.3 (6.3–34.3)	0.001
L $\rightarrow$ S <sub>max/min ratio</sub>	1.22 (1.0–2.1)	1.18 (1.0–1.6)	0.02
L $\rightarrow$ S $\Delta$ BP <sub>sys</sub> (mmHg)	-5.8 $\pm$ 15.5	-9.4 $\pm$ 13.5	0.12

Data are means  $\pm$  SD with *P* values calculated by an unpaired *t* test, medians (ranges) with *P* values calculated by the Mann-Whitney *U* test, or %. *P* values for nominal variables are calculated with the  $\chi^2$  test (for glucose tolerance categories,  $\chi^2$  test for trend). Albuminuria is the albumin-to-creatinine ratio  $\geq 3.0$  mg/mmol in an early morning spot urine sample. Mean arterial blood pressure is diastolic blood pressure + (0.33  $\times$  pulse pressure). Actual hypertension is systolic blood pressure  $\geq 160$  mmHg and/or diastolic blood pressure  $\geq 95$  mmHg. Glomerular filtration rate was calculated with the formula of Levey et al. (17). For explanation of autonomic function tests, see RESEARCH DESIGN AND METHODS.

analyzed as a single group. The analyses suggested that the association between CAFS and albuminuria was stronger in subjects with IGT or type 2 diabetes than in NGT subjects (Table 3). Additional adjustment for HbA<sub>1c</sub> or fasting glucose levels in the IGT and type 2 diabetes group did not materially affect these results (data not shown). Adjustment solely for age rather than for all of the stratification variables (model 1) was also performed. The odds ratios then were 1.18 (1.04–1.34) for all subjects, 1.02 (0.80–1.31) for the NGT group, and 1.19 (1.03–1.38) for the IGT and type 2 diabetes group, which indicates that the effect of adjustment for the stratification variables, especially in the NGT group, is explained mainly by the effect of age.

Note that we performed 2 measurements reflecting mainly parasympathetic function, 1 that reflects mainly sympathetic function, and 1 that combines parasympa-

thetic and sympathetic function. Combining the 4 autonomic function measures in 1 score carries the risk of unbalanced weighting of parasympathetic and sympathetic autonomic function. Therefore, we also constructed an alternative autonomic function score by combining the EIHR<sub>diff</sub> (mainly parasympathetic) and the L $\rightarrow$ S $\Delta$ BP<sub>sys</sub> (mainly sympathetic) in a similar manner as was done for the CAFS. The EIHR<sub>diff</sub> was chosen as the parasympathetic test because it showed the lowest correlation with the L $\rightarrow$ S $\Delta$ BP<sub>sys</sub>, indicating minimal collinearity. This alternative score resulted in odds ratios that were virtually identical to those reported for the CAFS in Table 3 (data not shown).

No evidence of an interactive effect between any measure of blood pressure and the CAFS regarding the occurrence of albuminuria was found. Likewise, we found no evidence of interaction between CAFS and age, waist-to-hip ratio, or protein

**Table 2—Mean CAFS in normoalbuminuric and albuminuric subjects**

	Normoalbuminuric subjects	Albuminuric subjects	P
All	5.9 (495)	7.5 (41)	<0.001
NGT	5.4 (246)	6.4 (10)	0.15
IGT	6.0 (133)	7.5 (10)	0.10
Type 2 diabetes	6.9 (116)	8.0 (21)	0.08
No actual hypertension	5.8 (428)	7.4 (23)	0.006
Actual hypertension	6.4 (67)	7.6 (18)	0.10

Data in parentheses indicate *n*. Actual hypertension was defined as systolic blood pressure  $\geq 160$  mmHg and/or diastolic blood pressure  $\geq 95$  mmHg with or without use of antihypertensive medication. All *P* values were calculated with the unpaired *t* test.

intake. Reanalysis of the data with albumin-to-creatinine ratios of 2.0 and 2.5 as lower cutoffs for albuminuria had trivial effects on the outcomes, as did exclusion of subjects with leukocyturia (*n* = 121) and exclusion of the 3 subjects with macroalbuminuria. If hypertension defined as systolic and diastolic blood pressure levels of  $\geq 160$  and/or  $\geq 95$  mmHg, respectively, and/or use of antihypertensive medication was entered instead of actual hypertension, then the results as presented in Table 3 were essentially identical (data not shown). Also, exclusion of subjects with actual hypertension from the analyses had trivial effects on the results. Finally, replacing waist-to-hip ratio with BMI did not change the outcome of any of the multivariate analyses.

A total of 192 subjects met the criteria for CVD&D. After adjustment for CVD&D in the IGT and type 2 diabetes subgroup, the odds ratio for the CAFS decreased slightly to 1.16 (1.00–1.34) and 1.14 (0.98–1.34) in models 1 and 2, respectively (from 1.20 and 1.19, respectively [Table 3]).

**CONCLUSIONS** — This study demonstrates that cardiovascular autonomic function is related to the presence of albuminuria in an age-, sex-, and glucose tolerance-stratified random sample of an elderly Caucasian population. This association was independent of other determinants of albuminuria in the Hoorn Study population (e.g., glucose tolerance, blood pressure, waist-to-hip ratio, protein intake, and homocysteine [21,22]) except for the fact that it was observed more clearly in subjects with IGT or diabetes than in NGT subjects. In subjects with NGT, the apparent correlation between autonomic function and albuminuria was almost fully explained by the effect of age, which was not an evident confounder of this correlation in IGT and type 2 diabetes. The subgroup analyses should, however, be interpreted

with some caution because they apply to a much smaller number of subjects, and the CIs of the subgroups show overlap.

Although the definitions of autonomic dysfunction are not uniform, the overall reported prevalence of autonomic dysfunction is ~7% in newly diagnosed type 2 diabetes (23). Autonomic dysfunction appears to be associated with age (11), sex (24), weight (9,13), blood pressure (9,11), fasting insulin level (9,24), glycemic control (13), and duration of type 2 diabetes (13,24). As for type 2 diabetes-related microvascular complications, several reports have suggested an association between autonomic dysfunction and retinopathy (11,13,25). Some studies have examined autonomic cardiovascular function in detail in relation to albuminuria in type 2 diabetes. An early study found higher urinary albumin excretion rates in patients with impaired HR variability, which is a feature of cardiovascular autonomic failure (9). Another report indicated that autonomic cardiovascular dysfunction measured with only a single test (deep breathing) was related to macroalbuminuria in a large (*n* = 949) sample of rela-

tively young and strictly hypertensive patients (13). In this study, normo- and microalbuminuric subjects were taken as a single control group. An abnormal day–night blood pressure pattern was related to abnormal autonomic function measures and proteinuria in a study involving 76 subjects (10). Two small studies have shown conflicting results (11,12). Finally, a recent study found that albuminuria was related to Valsalva and breathing ratios only in patients with a duration of type 2 diabetes of >1 year (14). Compared with these previous studies, our study has the advantage of being large (which allows the use of multivariate tests), being truly population based, and including patients with NGT and IGT and with normal and increased blood pressure levels. Also, we were able to include 4 different measures of autonomic function. Previous studies used less extensive autonomic function tests except for 1 (11) that unfortunately included only 9 type 2 diabetic patients with albuminuria.

Two explanations exist for a possible causal relationship between cardiovascular autonomic function and urinary albumin excretion. First, a reduced nightly drop in blood pressure, which is a feature of autonomic dysfunction not only in type 1 diabetes but also in type 2 diabetes (26), may result in albuminuria. Second, a disturbance in glomerular arteriolar autoregulation may result in an inability of the glomerular apparatus to counteract hyperglycemia-associated glomerular hypertension and hyperfiltration (27). Glomerular hyperfiltration has been reported to occur in type 2 diabetes but has also been found in subjects with IGT (28). Our findings may indicate that IGT- and type 2 diabetes-associated effects on glomerular

**Table 3—Multiple logistical regression analyses with albuminuria as the dependent variable and the CAFS as the independent variable**

	Crude model	Model 1	Model 2
All subjects	1.22 (1.08–1.37)	1.15 (1.01–1.30)	1.14 (1.00–1.31)
NGT	1.14 (0.91–1.44)	1.03 (0.81–1.32)	1.05 (0.81–1.38)
IGT and type 2 diabetes	1.20 (1.04–1.39)	1.20 (1.04–1.39)	1.19 (1.02–1.39)

Data are odds ratios (95% CIs). The odds ratio is expressed for each point increase in the autonomic cardiovascular function score. Separate results are given for the complete study group and for the substrata of NGT and IGT plus type 2 diabetes. For all subjects, model 1 included adjustment for age, sex, and glucose tolerance category. Model 2 was the same as model 1 plus adjustment for actual hypertension, waist-to-hip ratio, triglycerides, and HDL cholesterol. In substrata of glucose tolerance, models 1 and 2 were identical but without glucose tolerance as a covariate. Additional adjustment for glomerular filtration rate, total protein intake, homocysteine, and current or ever smoking provided results virtually identical to model 2 in both the entire group and each of the substratum analyses (data not shown).

hemodynamics may be required for autonomic cardiovascular dysfunction to reveal its effect on albuminuria.

Another possibility is that the association we found is not causal but simply reflects an association between 2 diabetic complications caused by largely the same set of risk factors. However, if this is the case, then the introduction of these risk factors as additional independent variables on top of the crude model with only the CAFS would have introduced significant colinearity among the independent variables. This would normally be reflected by a marked decrease in the odds ratio and/or in the partial  $R^2$  for the CAFS, which was not seen (the partial  $R^2$  for the CAFS in the IGT and type 2 diabetes stratum was 0.15 in the crude model, 0.14 in model 1, and 0.12 in model 2 [Table 3]). Even if the association at issue does not reflect causality between albuminuria and cardiovascular autonomic function, our results may still be important in the context of understanding why albuminuria predicts mortality in type 2 diabetes. Many believe that this prognostic significance of albuminuria is explained by its association with several atherosclerotic risk factors, including hypertension, dyslipidemia, smoking, and poor glycemic control. However, albuminuria still predicts cardiovascular events after adjustment for these risk factors (29). Two explanations may exist for this. First, albuminuria may reflect early generalized vascular/endothelial damage (1,30). Alternatively, cardiovascular risk factors other than those commonly studied may hide beneath the independent prognostic significance of albuminuria, and cardiovascular autonomic neuropathy may be such a risk factor (24,31). In fact, the only study we know of that has included both albuminuria and cardiovascular neuropathy as predictors of cardiovascular disease in a multivariate analysis found that albuminuria was no longer a prognostic factor after adjusting for the presence of autonomic neuropathy (3).

We did not regard the autonomic test results as dichotomous variables for "normal" and "abnormal" results. We chose this approach because abnormal values for autonomic function tests have been defined on the basis of statistical abnormality in a healthy control population rather than on the basis of pathophysiological alterations. No evidence exists to indicate that these statistically abnormal results have a pathophysiological meaning in the context of a possible association with albuminuria. Also, we avoided the use of age-corrected autonomic

function measures. Albuminuria increases with age, and whether this effect is (partially) mediated by deterioration in autonomic function is not known. However, should this be the case, then removing the age effect from the autonomic function variable by using age-corrected values would inadvertently exclude autonomic function as a determinant of albuminuria. Adjusting for age in the multivariate model by including it as a separate independent variable is, in our view, the best option. A final possible concern is whether combining several autonomic function measures in a single score is legitimate. We constructed the CAFS with the purpose of enhancing power because a battery of autonomic function measures will by definition show a higher reproducibility than any single measurement. Also, the 4 measures are thought to convey distinct information about cardiovascular autonomic function, although the specificity for the specific subtype of autonomic function (sympathetic vs. parasympathetic) should not be overestimated (19,20). Still, the contention of supplemental information from the 4 tests is supported by generally poor correlations that we found between the 4 tests. Except for a correlation of 0.60 between the  $L \rightarrow S\Delta HR_{\max}$  and the  $L \rightarrow S_{\max/\min \text{ ratio}}^{\text{diff}}$ , all correlations were  $<0.40$ . Even the  $ELHR_{\text{diff}}$  and the  $L \rightarrow S\Delta HR_{\max}$ , which are both considered to reflect mainly parasympathetic function, correlated poorly (correlation coefficient 0.37) and were quite differently associated with albuminuria (Table 1). Even so, giving equal weight to measures that overlap even partially could possibly result in a CAFS that does not give a balanced impression of overall autonomic function. In our view, the virtually identical results that were obtained using an alternative score that combined a mainly parasympathetic with an uncorrelated mainly sympathetic measurement make the possibility that the CAFS was heavily unbalanced unlikely.

In conclusion, our study shows that autonomic cardiovascular function is associated with the occurrence of albuminuria, especially in subjects with IGT or type 2 diabetes, and that this association is independent of age, blood pressure, and other previously identified determinants of albuminuria in the same study population.

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